refer to granules within the trophosome cells having the dimensions of bacteria.

From the standpoint of the trophic structure of the vent community, the precise localization of the sulfur metabolism and Calvin-Benson cycle enzymes within the trophosome is of less importance than the fact that *R. pachyptila* may represent the first example of an autotrophic animal situated at the base of a food chain.

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Hydrothermal Vent Clam and Tube Worm $^{13}C/^{12}C$: Further Evidence of Nonphotosynthetic Food Sources

Abstract. *The stable carbon isotope ratios in clam mantle tissues taken from both Galápagos and 21°N hydrothermal vent sites were similar to the unusually low ratios of carbon-13 to carbon-12 previously reported for a Galápagos hydrothermal vent mussel. In marked contrast to these bivalves, vestimentiferan worm tissues from a Galápagos vent had isotope ratios that were higher than those of open ocean biota. These observations suggest that more than one nonpelagic and nonphotosynthetic carbon fixation pathway is of nutritional importance to vent animals, and that at least one of these pathways is common to two geographically separated vent sites.*

After dense animal communities were discovered near Pacific Ocean hydrothermal vents (*1*), on-site microbial chemosynthesis was implicated as the primary source of reduced carbon for these organisms. The locally large standing crop and apparent rapid growth (*2*) of some of these animals require a food base that is much more abundant than is normally available to deep-sea benthos. These circumstances, however, do not preclude the possibility that the relatively sparse food resource sedimenting from the ocean's euphotic zone is being physically concentrated or entrained by advective currents near the vents (*3*). Still, high rates of microbial carbon fixation can occur in vent waters (*4*), and this chemosynthetic primary production could conceivably be an important source of energy and biomass for higher trophic levels. Support for the concept that vent animals do not rely on imported, photosynthetically derived food sources was found in the striking dissimilarity between the $^{13}C/^{12}C$ of a vent mytilid mussel and the $^{13}C/^{12}C$ of nonvent marine organisms (*5*). Further isotopic evidence for vent food web autochthony is presented in this report.

Portions of mantle tissue from whole, frozen vesicomyid clams, *Calyptogena magnifica* Boss and Turner, collected by the *Alvin* submarine from the Rose Garden site (Galápagos Rift) and from the 21°N site (East Pacific Rise), were obtained from Dr. George Somero, Scripps Institution of Oceanography. Also removed were samples of frozen vestimentum and trophosome of a vestimentiferan worm, *Riftia pachyptila* Jones, originally collected from Rose Garden. All of the above samples were dried for several days at 60°C and then ground or pulverized. A 50-mg portion of the dried, ground vestimentum was submerged in 8 percent HCl and the solution was then gently heated to dryness. Subsamples (5 to 10 mg) of all of the above tissues were then combusted and the resultant CO_2 was purified, collected, and isotopically analyzed by previously described methods (*6*). The $^{13}C/^{12}C$ of each sample is reported as $\delta^{13}C$, the relative per mil difference between the $^{13}C/^{12}C$ of the sample and the $^{13}C/^{12}C$ of the PDB carbonate standard (*7*).

The $\delta^{13}C$ of clam mantle tissues from both Galápagos and 21°N vents are quite similar to the previously reported $\delta^{13}C$ of a Galápagos vent mussel (Table 1). This earlier study (*5*) pointed out that such $\delta^{13}C$ values are lower than those of animals sampled from other marine envi-

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ronments. Pressure or depth effects apparently cannot explain the ^{13}C-depleted condition of these vent animals because the $\delta^{13}C$ of bathypelagic organisms and detritus, taken from depths comparable to the vents, are usually similar to the $\delta^{13}C$ of the organic material existing near the ocean surface (*8*). Metabolic isotope fractionation effects, which would significantly lower a clam's or mussel's $\delta^{13}C$ below that of its food source, are unlikely in view of controlled invertebrate experimentation (*9, 10*) and analyses of naturally occurring bivalve specimens (*5, 10, 11*). It is therefore concluded that the consistently low $\delta^{13}C$ of vent bivalves results from the utilization of food sources whose derivation (and hence, stable carbon isotope ratio) is unlike that of the organic material found in the overlying ocean.

As to the nature of this isotopically different food source, chemoautotrophic bacteria are present in vent waters (*4, 12*) and seem likely food items for filter feeding animals. A limited number of analyses suggest that some chemoautotrophic bacteria preferentially fix $^{12}CO_2$ to a greater extent than do photoautotrophs, thus producing biomass whose $\delta^{13}C$ can be lower than that of available inorganic carbon by 30 per mil or more (*13, 14*). It is therefore possible to explain the approximate 28 per mil difference between bivalve $\delta^{13}C$ and the $\delta^{13}C$ of vent water ΣCO_2, ≥ -4 per mil (*15*), as entirely due to carbon isotope fractionation incurred during chemosynthesis of organic matter and preservation of the resultant isotope abundances in higher trophic level biomass. Microbial fixation of carbon sources other than CO_2 (*16*), as well as carbon fixation driven by the oxidation of elements or compounds other than the most obvious one, hydrogen sulfide, may also merit consideration. At present, the carbon isotope abundances produced by chemoautotrophs are in almost all cases unknown.

That more than one type of autotrophy exists at the vents is indicated by the relatively high $\delta^{13}C$ of *R. pachyptila* tissue (Table 1). By comparison with acidified worm tissues (Table 1), these elevated $\delta^{13}C$ values cannot be the result of ^{13}C-rich carbonate contamination. Although littoral animal $\delta^{13}C$ values less negative than -15 per mil are not uncommon (*10, 11, 17*), such values are interpreted as resulting from the utilization of near-shore C_4 plant material as a food source. The C_4 plants produce biomass whose $\delta^{13}C$ values typically range from -6 to -15 per mil (*18*). It is not evident from $\delta^{13}C$ values of open-ocean animals, plants, or detritus (*8*) that ^{13}C-enriched material produced by C_4 photoautotrophs is an important food source for pelagic organisms and, subsequently, deep-sea benthos. Therefore, possessing an internal autotrophic capability (*19, 20*) and evidently lacking a way of ingesting particulate food sources (*21*), vestimentiferan worms found at the Galápagos and other vent sites apparently rely on organic carbon produced (i) within their bodies and (ii) by pathways that fractionate carbon isotopes in a way different from those pathways supplying hydrothermal vent bivalves.

Table 1. The replicate $\delta^{13}C$ values of vesicomyid clam and vestimentiferan worm tissues taken from the hydrothermal vent sites indicated. For comparison, the $\delta^{13}C$ ranges of previously analyzed marine organisms are also listed.

Animal	Hydrothermal vent site	$\delta^{13}C$, per mil (± 0.2 per mil)
Clam (mantle)	21°N (East Pacific Rise)	−32.6, −32.7
Clam (mantle)	Rose Garden (Galápagos)	−32.0, −32.1
Mussel (foot and mantle)*	Clambake 1 (Galápagos)	−32.7 to −33.6
Vestimentiferan worm	Rose Garden (Galápagos)	
Vestimentum (muscle)		−10.8, −10.8, −11.0
Vestimentum (acid treated)		−10.9, −11.0
Trophosome		−10.9, −11.1
	Nonvent data	
Nonvent marine organisms†	Temperate and tropical waters	−8 to −25

*Data from (5). †Data from (5, 8, 10, 11, 14, 17).

The presence in *R. pachyptila* of carbon isotope abundances resembling those of C_4 plants—in spite of the apparent lack of a crucial C_4 enzyme in the worm's autotrophic trophosome (*20*)—presents an anomaly also recently found in the marine seagrass *Thalassia testudinum*. In this C_3 plant, $\delta^{13}C$ values also near -11 per mil are explained by Benedict *et al.* (*22*) as arising from a CO_2 limitation that occurs within the plant forcing the carboxylation of ribulose bisphosphate to be less isotopically selective in the use of $^{13}CO_2$ or $^{12}CO_2$. Consequently, unlike other C_3 autotrophs, relatively little isotopic fractionation is exhibited between the inorganic carbon available to this plant, -10 per mil, and the bulk organic carbon the plant produces, -11 per mil. A limited internal supply of CO_2 relative to internal demand by carbon-fixing processes may then also explain the unexpectedly high $\delta^{13}C$ values observed in *R. pachyptila*.

In any case, the unusual stable carbon isotope abundances found in Galápagos and 21°N hydrothermal vent animals indicate that their primary sources of organic carbon are unlike those commonly used by marine animals. On-site carbon fixation, at the expense of chemical rather than light energy, is apparently the ultimate source of this isotopically distinctive animal biomass. Furthermore, the large isotopic dissimilarity between vestimentiferan worm and bivalve tissues suggests that at least two different, locally produced food sources are being utilized by vent organisms. Use of autochthonous sources of food is also borne out by recent measurements of ^{15}N (*23*) and ^{14}C (*24*) natural abundances in vent animals.

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Prokaryotic Cells in the Hydrothermal Vent Tube Worm *Riftia pachyptila* Jones: Possible Chemoautotrophic Symbionts

Abstract. *The existence of a symbiotic association between vestimentiferan tube worms from deep-sea hydrothermal vents and chemoautotrophic sulfur-oxidizing prokaryotes, based on histological and enzymatic evidence, is suggested.*

A major recent biological discovery is that of the dense benthic animal populations clustered tightly around the newly explored deep sea hydrothermal vents at the Galápagos Rift and East Pacific Rise ocean spreading centers (*1, 2*). The primary or sole base of the food chain in these communities appears to be the chemoautotrophic production of bacterial biomass with hydrogen sulfide emitted from the vents as the geothermic source of energy (*3*).

The most conspicuous animal of these vent communities is the large red-plumed vestimentiferan tube worm, *Riftia pachyptila* Jones (*4*), of the phylum Pogonophora (*5*). Observations from the submersible *Alvin* indicate that the tubes of *Riftia* are attached to rocks situated directly in the flow of sulfide-rich seawater from the vent (H_2S, up to 160 μM) (*2*). *Riftia* is superficially similar but much larger (up to 1.5 m in length and a diameter of 38 mm) than other related benthic pogonophorans (*4*). The most striking feature of this phylum is the lack of mouth and gut. *Riftia*'s large size is astonishing because speculation on the mode of nutrition of the Pogonophora has centered on the uptake of dissolved organic material via the epidermis (*6*). However, uptake rates of dissolved amino acids at ambient concentrations by the pogonophoran *Siboglinum fiordicum* were shown to be insufficient to account for the animal's metabolic requirements (*7*). Thus, the mechanism of nutrition of this group remains unclear.

Specimens of *Riftia* were collected at a number of geothermal vents in the Galápagos Rift and East Pacific Rise. The body of the animal consists of four regions (Fig. 1A). The cavity of the trunk, the third and most extensive region (at least 50 percent of the total length in postjuvenile specimens), is occupied by the gonads and the trophosome. The latter, of irregular and variable development along its length, is compact, of many lobules, and is well-supplied with vascular elements. Prior to this study the function of the trophosome in vestimentiferans was unknown. In *Lamellibrachia luymesi* it was suggested to serve as a source of nutrition for developing sperm or as a detoxifying organ (*8, 9*). In 21 of 31 specimens of *Riftia* examined, crystals of elemental sulfur up to 100 μm in size were found within the trophosomal tissue (*10*). This observation suggested a capacity for using the chemoautotrophic oxidation of sulfide as an internal source of nutrition. We report here a number of subsequent observations demonstrating the presence of prokaryotic cells within the trophosomal tissue of *Riftia pachyptila* Jones.

In stained paraffin sections the trophosomal tissue is granular (Fig. 1A) (*11*). The granules are usually aggregated in lobelike accumulations. There are few nuclei present, and the majority of these are associated with blood vessels and the squamouslike covering of the lobular surfaces. Juveniles as small as 1.44 mm long and 0.33 mm in diameter have a trophosome identical in appearance to that of post-juveniles (Fig. 1A). A trophosomal tissue homogenate stained with 4′,6-diamidino-2-phenylindole (a specific and sensitive DNA stain) and examined with epi-fluorescence microscopy (*12*) revealed that the morphologically distinct granules (3 to 5 μm in diameter) uniformly produced a brilliant blue fluorescence. This is interpreted as their being either prokaryotic cells or eukaryotic organelles (such as mitochondria). Direct counts of this trophosome homogenate indicated that there were 3.7×10^9 cells measuring 3.0 μm or more per gram of tissue (wet weight) (*13*).

The trophosomal tissue available for examination by electron microscopy was collected at the Rose Garden geothermal vent (*14*) and had been fixed in 5 percent Formalin (in seawater) as a general preservative. Consequently, the fixation for transmission electron microscopy is not of the highest quality but is sufficient to resolve important structural features. Scanning electron microscopy revealed that the characteristic lobes of the trophosomal tissue consist of densely packed spherical bodies (Fig. 1B). Transmission electron microscopy (TEM) of the same tissue (*15*) indicated that these bodies are prokaryotic cells varying in size between 3 and 5 μm (mean = 4.20 ± 0.64 μm; n = 16) and having a cell wall resembling that of gram-negative bacteria (Fig. 1C). To date we have been unable to determine if the prokaryotic cells are located within or outside the trophosomal cells.

The presence of lipopolysaccharide (LPS), a compound characteristic of the outer cell wall of gram-negative bacteria, was confirmed in frozen trophosomal tissue by the *Limulus* amoebocyte lysate test (*16*). The result of the assay was strongly positive (0.8 μg of LPS per milligram of wet tissue) indicating the presence of a large population of gram-negative prokaryotic cells far in excess of what could be attributed to bacterial contamination of the frozen sample.

Felbeck (*17*) found in the trophosomal tissue high activities of enzymes used in generating adenosine triphosphate (ATP) from the oxidation of reduced sulfur compounds, that is, thiosulfate sulfurtransferase (rhodanese), APS reductase, and ATP sulfurylase. In addition, high activities of RuBP carboxylase and ribulose 5-phosphate kinase (enzymes of the Calvin-Benson cycle of CO_2 fixation) have been measured in the trophosome in activities comparable to those of spinach leaves (*17*). The prokaryotic cells make up a major portion of the trophosome in *Riftia*. This suggests strongly that they are responsible for these enzymatic activities and are symbiotic chemoautotrophic bacteria that are capable of generating ATP by way of sulfide oxidation and reducing CO_2 to organic matter. Preliminary studies by Rau (*18*) on $^{13}C/^{12}C$ ratios in *Riftia* lend support to

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